PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of

Gerald WALKER, et al.

Appln. No.: 10/563,594

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Examiner: Jonathan C. Langman

For: COMPOSITE MATERIAL HAVING THE APPEARANCE OF NATURAL STONE

DECLARATION UNDER 37 C.F.R. § 1.132

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, **Raúl Pozas Bravo**, hereby declare and state:

THAT I am a citizen of Spain;

THAT I have received the degree of Chemistry in University of Jaen from Spain and the Ph.D in Material Science in University of Sevilla from Spain.

THAT I have been employed by COSENTINO since 2007, where I hold a position as researcher in R&D department, with responsibility for the development of nanotechnology research projects;

THAT I am familiar with the Office Action dated January 21, 2010 and the rejections set forth therein;

THAT I am familiar with U.S. Patent 6,663,877 to Appleton et al. ("Appleton");

THAT, as a means to demonstrate the superior antimicrobial properties and migratory abilities of the composite material described in the claims of the above-noted application, the following study were made and supported by Universidad Autónoma de Barcelona (UAB), including several experiments based on the synthesis of materials that were conducted by me or under my supervision. The UAB report is also annexed to this document.

1. INTRODUCTION

Appleton discloses that the effectiveness of solid surface materials (i.e. Corian®) degrades very easily over time, and that this effectiveness could be re-established by abrading the outer surface of the various solid surface materials. *See* column 9 of Appleton, line 60-65.

In Appleton, to demonstrate this antibacterial degradation, samples of Corian® with silver additives were subjected to shaking for 24 hours in 75 mL of different types of household cleaning products, and the resulting antibacterial effectiveness was determined. After shaking with the cleaners and before testing for antibacterial effectiveness, the samples were extensively washed with deionized water in order to remove any antibacterial effect of the cleaners. *See* Id.

In Examples 25-36 of Appleton, the household cleaners reduced the antibacterial effectiveness of Corian®. *See* Table 4 of Appleton. For example, the antimicrobial effectiveness decreased from 5.90 to 1.83 after 24 hours in contact with *E. Coli*. *See* Id.

In my opinion, it is well-known that a reduction of the antibacterial effectiveness (deactivation) in such materials is attributed to the consumption of surface biocides. This consumption is caused by the solubilization or migration of the antimicrobial active agents (silver cations) in water.

In contrast to the Appleton agglomerates, the present quartz agglomerate surfaces (the solid surface materials claimed in the above-noted application) can preserve the antibacterial efficiency after multiple surface treatments, and thus demonstrate the unique durability of the antibacterial properties of the present agglomerates.

In my opinion, this behavior takes place independently of the nature of biocides (organic or inorganic) incorporated in the agglomerate and as consequence, it is not necessary to re-establish the antibacterial effectiveness by abrading the surface, as is required for the agglomerates of Appleton.

Furthermore, the below experiments are evidence that organic biocides migrate from the inner to the upper part of the presently claimed quartz agglomerates, which provide the presently claimed agglomerates with an antibacterial efficiency that is both durable and effective over time.

In my opinion, these cumulative results suggest that the present quartz agglomerates have antibacterial properties that are more efficient, more durable, and last for a longer period of time than the Appleton agglomerates.

In my opinion, this advantageous behavior is attributed to the design of the present agglomerates, which allows the antimicrobial agents to disperse through the polyester resin and mineral filler.

2. EXPERIMENTAL

2.1. Preparation of quartz agglomerates with antimicrobial properties

Several slabs were manufactured with antibacterial properties with the following composition:

- Micronized silica: 28%
- Ground quartz: 61%
- Unsaturated polyester resin binder: 10.5% containing:
    - a catalyst,
    - an accelerator,
    - an adhesion promoter and optionally
    - a coloring agent (white)

- an organic biocide (triclosan or zinc pyrithione) added in the concentration range between 400 and 1,000 ppm or an inorganic biocide (sodium phosphate glass based silver) with a concentration of 1,000 ppm.

The percentages reflect weight percent based on the total weight of the composition, including resin, filler, pigments, etc.

This procedure involves a first step wherein the organic or inorganic biocide is dispersed (zinc pyrithione or silver) or solubilized (triclosan) and stabilized in an unsaturated polyester resin (with higher viscosity than that used in the process) forming a stable biocidal concentrate suspension (40% in weight for zinc pyrithione and silver) or a stable biocidal dissolution (33% in weight for triclosan).

For this purpose, some wetting agents compatible with biocide and the polyester resin were added to allow the biocidal particles to disperse in the resin binder. These biocidal suspensions were then added and dispersed into the rest of resin binder along with the rest of additives including a catalyst, an accelerator, an adhesion promoter and the coloring agent. Finally the resulting mixture (called a resin binder) was mixed along with the micronized silica and ground quartz and the final quartz agglomerate was synthesized according to conventional procedures.

While Appleton does not give any details regarding how biocides are added to their agglomerates, in view of the low durability for the antimicrobial efficiency of the final product of Appleton, it is my opinion that in Appleton, biocides are added along with the mineral filler so that a low dispersion grade is reached. This low dispersion grade explains why the Appleton composite behaves differently than the present agglomerates, shown below.

2.2. Biocide consumption on the test specimens

In order to evaluate whether the resulting antibacterial effectiveness is affected in the same grade as Appleton, biocidal surface consumption was measured in three different ways:

i) by immersion of the test specimens in 100 mL of water at 40°C during 24 hours,

ii) by repeated analysis by ISO 22196 (attached herewith) of the same samples (repeated inoculation of the analyzed surfaces), and

iii) by immersion and shaking of the test specimens in 75 mL of water containing a typical household cleaner during 24 hours. After shaking with the cleaners and before testing for antibacterial effectiveness, the samples were extensively washed with deionized water (6 times x 100 mL and a final washing with 100 mL water in the presence of stirring) in order to remove any antibacterial effect of the cleaners.

The antibacterial efficiency of the resulting surfaces tested was evaluated by the procedure described in section 2.1 prior to and after each treatment for, purposes of comparison.

2.3. Measurement of antibacterial activity on quartz agglomerates surfaces

The measurement of the antimicrobial efficiency against *S. aureus* and *E. coli* on the quartz agglomerate surfaces carried out herein is based on International Standard ISO 22196:2007(E), adapted to the measurement of agglomerate stone surfaces following the next steps.

The antibacterial activity is defined as the difference in the logarithm of the viable cell count found on an antibacterial-treated product and an untreated product after inoculation with an incubation of bacteria.

The test bacteria were *S. aureus* and *E. coli*.

2.3.1. Preparation of test specimens

Testing was carried out on three specimens from each treated test material, as well as six specimens of the untreated material. Half of the untreated test specimens were used to measure viable cells immediately after inoculation and half were used to measure viable cells after incubation for 24 h.

50 mm x 50 mm (and 12 mm in thickness) specimens of the treated and untreated test materials were prepared. Test specimens were sterilized prior to testing by autoclaving at 121°C during 2 x 15 min.

2.3.2. Preparation of test inoculums

Using a sterile inoculating loop, one loop of the pre-incubated test bacteria was transferred as specified in section 7.1 of ISO 22196 into a small amount of 1/500 NB prepared in accordance with section 4.2.3.2 in ISO 22196. The test bacteria was evenly dispersed, and the number of bacteria was estimated using direct microscopic observation and a counting chamber or another appropriate method (e.g. spectrophotometrically). The suspension was diluted with 1/500 NB, as appropriate for the estimated bacterial concentration, to obtain a bacterial concentration that was between 1.0×10^6 cells/mL and 4.0×10^6 cells/mL. This solution was used as the test inoculums.

2.3.3. Inoculation of test specimens

The surface to be tested is the exposed outer polished surface of the product. Each test specimen prepared in 7.2 was placed into a separate sterile Petri dish with the test surface uppermost. 0.1 mL of the test inoculums was pipetted onto each test surface. Each test surface containing inoculum was covered with a piece of film that measures 40 mm x 40 mm. The films were pressed so that the test inoculum spreads to the edges such that the test inoculum does not leak beyond the edges of the film. After the specimen has been inoculated and the cover film applied, the lid of the Petri dishes were put back on. As consequence, a number of bacteria between 6.2×10^3 cells/cm^2 and 2.5×10^4 cells/cm^2 were inoculated on the surface of the product such as indicated in ISO 22106.

2.3.4. Incubation of the inoculated test specimens

The Petri dishes containing the inoculated test specimens were incubated (including half of the untreated test specimens) at a temperature of 35°C and a relative humidity of not less than 90% for 24 h. The antibacterial effectiveness of a product was evaluated based on the value of the antibacterial activity obtained from the test at the incubation temperature specified.

2.3.5. Recovery of bacteria from test specimens

2.3.5.1. Test specimens immediately after inoculation

Immediately after inoculation, process half of the untreated test specimens were processed by adding 10 mL of either SCDLP broth (see 4.2.3.6 section in ISO 22196) to the Petri dish

containing the test specimens. This value was used to determine the recovery rate of the bacteria from the test specimens under investigation. In order to improve the recovery, mechanical agitation (vortexing) of the test specimens was required.

2.3.5.2. Test specimens after incubation

After incubation, the remaining test specimens were processed as mentioned above. The viable bacteria recovered from the test specimen were counted.

2.3.6. Determining the viable bacteria count by the pour plate culture method

Viable bacteria were enumerated by performing 10-fold serial dilutions of the SCDLP in phosphate-buffered physiological saline (see section 4.2.3.6 in ISO 22196). 1 mL of each solution, as well as 1 mL of the SCDLP recovered from the test specimen was placed into separate sterile Petri dishes. 15 mL of plate count agar was poured into each Petri dish and swirled gently to disperse the bacteria. Plating was performed in duplicate. The Petri dishes were inverted and incubated at 35°C for 40 h to 48 h.

After incubation, the number of colonies was counted in the Petri dishes containing 30 to 300 colonies. For each series dilution, the number of colonies recovered was counted, as well as the dilution factor for the plates used for counting. If the number of colonies in the plates containing the 1 mL aliquots of SCDLP was less than 30, then the number of colonies in these plates was counted and recorded. If there were no colonies recovered in any of the agar plates in the dilution series, the number of colonies was recorded as "< 1."

2.3.7. Calculation of the antibacterial activity

The antibacterial activity against S. Aureus was calculated using the following Equation:

$R = (U_t - U_0) - (A_t - U_0) - (A_t - U_0) = U_t - A_t$

Where

R is the antibacterial activity

U_0 is the average of the common logarithm of the number of viable bacteria, in cells/cm^2, recovered from the untreated test specimens immediately after inoculation;

U_t is the average of the common logarithm of the number of viable bacteria, in cells/cm^2, recovered from the untreated test specimens after 24 h;

A_t is the average of the common logarithm of the number of viable bacteria, in cells/cm^2, recovered from the treated test specimens after 24 h.

2.4. Migration of Triclosan contained into the quartz agglomerate

The procedure followed to evaluate the actual migration of triclosan from inner to upper part of the agglomerate was carried out by chemical analysis by HPLC of the agar as described in the attached protocol entitled "bacteriostatic protocol."

3. RESULTS AND DISCUSSION

3.1. Biocidal consumption on the test specimens by immersion in water at 40°C during 24 hours

The below table summarizes the antimicrobial activity of the present composite against *S. Aureus* determined for the test specimens containing different biocides substances, prior to and after its immersion in water at 40°C during 24 hours.

Biocide Concentration		**Antibacterial Activity (R) against *S. Aureus***	
Type of biocide incorporated in quartz agglomerate	**Biocide concentration (ppm)**	**Prior to immersion in water at 40°C/24 h**	**After immersion in water at 40°C/24 h**
0	0	= 0.0	= 0.0
Silver	1000	> 5.7	> 5.7
Triclosan	1000	= 1.8	= 2.2
Zinc Pyrithione	500	> 5.8	> 5.8
Zinc Pyrithione	1000	> 5.8	> 5.8

As can be seen in the above Table, the antibacterial activity of the quartz aggregate samples is preserved even after immersing the test specimens in water at 40ºC for 24 hours, which is indicative of durability and reflective of strong antibacterial performance. It should be noted that this behavior is not only observed when an organic biocide (triclosan, zinc pyrithione) is incorporated in the quartz agglomerate, but also when an inorganic compound (silver) is used.

These results are in contrast to the low antimicrobial life-span exhibited by the Corian® surfaces containing silver compounds (Novaron) and disclosed in the Appleton patent, which require re-establishing the antimicrobial effectiveness by abrading the outer surface when the surface is exposed to a deactivation process. Further, this different behavior in the two agglomerates can be explained in terms of the different biocidal dispersion grade in the final agglomerate such as it was discussed above in section 2.1.

In my opinion, the present quartz agglomerates have a higher antimicrobial life-span due to the predispersion carried out prior to the quartz agglomerate synthesis process.

3.2. <u>Biocidal consumption on the test specimens by repeated analysis by ISO 22196 of the same samples</u>

Another deactivation process based on the repeated inoculation with *S. Aureus* was tested. This process consisted of the application of repeated inoculations with *S. Aureus* on the quartz agglomerate in order to make a consumption of the antimicrobial agent present on the material surface which is used to act against bacteria, giving a reduction in its final antibacterial efficiency by decreasing the active agent concentration on the surface.

The inoculations were carried out by applying 0.4 mL of inoculums with a high concentration in *S. Aureus* (in the range of $10^7 - 10^8$ UFC/mL) in order to accelerate the deactivation process.

The below table summarizes the antimicrobial activity against *S. Aureus* determined for the test specimens containing different biocides substances, prior to and after its repeated inoculation with *S. Aureus*.

Biocide Concentration		**Antibacterial Activity (R) against *S. Aureus***					
Type of biocide incorporated in quartz agglomerate	**Biocide concentration (ppm)**	**As prepared**		**After one inoculation with *S. Aureus***		**After 5 inoculations with *S. Aureus***	
0	0	=	0.0	=	0.0	=	0.0
Silver	1000	>	5.7	>	5.7	>	5.5
Triclosan	1000	=	1.8	=	2.2	=	2.4
Zinc Pyrithione	500	>	5.8	>	5.8	>	5.3
Zinc Pyrithione	1000	>	5.8	>	5.8	>	6.2

It can be observed that the antibacterial activity is not altered by the repeated inoculation with *S. Aureus*. These results are in agreement with the behavior showed in section 2.1, showing the large durability for the antibacterial performance exhibited by the biocide modified quartz agglomerates surfaces. In this case, the high durability is again observed for the use of either an organic biocide (triclosan, zinc pyrithione) or an inorganic one (silver).

Furthermore, these results are in contrast to the low antimicrobial life-span of the antimicrobial effectiveness exhibited previously by the Corian® surfaces containing silver compounds (Novaron) and disclosed in the Appleton patent, which require re-establishing the antimicrobial effectiveness by abrading the outer surface when the surface is exposed to a deactivation process.

In contrast, for the composites disclosed in the above-noted application, the antimicrobial efficiency is advantageously maintained at length without requiring any surface treatment.

Thus, the present study tends to show that incorporating antibacterial additives (organic or inorganic) in quartz agglomerates result in a material with an added value in relation to the durability of the resulting properties.

3.3. Biocidal consumption on the test specimens by immersion and shaking in a household cleaner during 24 hours

The obtained results are illustrated in the following table:

Sample details			**Antibacterial Activity (R)**	
Type of biocide incorporated in quartz agglomerate	**Biocide concentration (ppm)**	**Microorganism**	**As Prepared**	**After household cleaner treatment for 24 h**
Silver	1000	*S. Aureus*	= 4.6	= 5.5
Silver	1000	*E. Coli*	> 7.1	> 7.1

The above table shows that the antibacterial activity of the present quartz agglomerates is maintained against both *S. aureus* and *E. coli* after household cleaner treatment for 24 h.

These results are evidence that the present agglomerates do not require any surface treatment to reestablish antimicrobial effectiveness, which is in direct contrast to the antimicrobial life-span of the Appleton agglomerates, which require re-establishing the antimicrobial effectiveness by abrading the outer surface when the surface is exposed to a deactivation process.

3.4. Triclosan migration from inner to upper parts of quartz agglomerates using inhibition halo testing

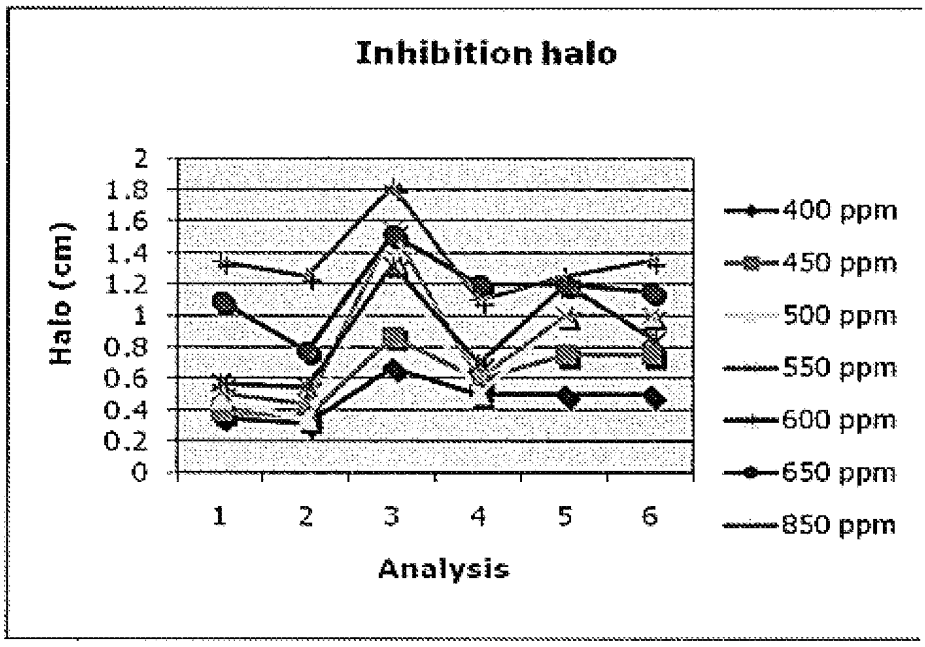
The protocol followed to analyze the samples is attached to the present Declaration, labeled as "Bacteriostatic Protocol and Durability of Hard Surfaces."

The triclosan removed from the surface in each inoculation step after 24 hours in contact with S. Aureus was determined by measuring the inhibition halo whose diameter is directly proportional to the triclosan concentration present in the agar medium.

The below Figure shows that halo length is higher for the sample containing higher triclosan concentration, such as it is expected. Furthermore, it should be noted that this parameter is also kept almost constant in each of the 15 steps comprising the analysis developed, independently of triclosan concentration present in the quartz agglomerate.

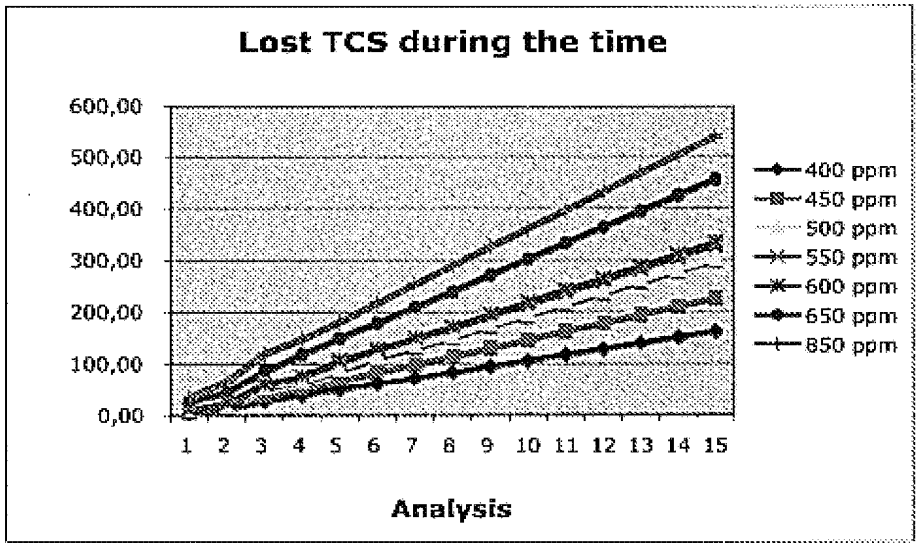
This is indicative of a slow and continuous migration of the organic molecule from the agglomerate surface up to the agar medium, giving an equivalent antibacterial effect in each step.

These results also show that triclosan is not consummated after 15 steps, in agreement with the high antibacterial lifespan detected in sections 3.1 and 3.2 and consumption of section 3.3.



The below figure shows the amount of accumulated triclosan lost during each step of the testing, which was determined by chemical analysis of the nutrient agar after exposing it to the agglomerate surface for 24 hours. As can be expected from the laws of diffusion, the removal rate of triclosan from the material to the agar medium (migration) was higher for the more concentrated samples. The rate lost remained almost constant during analysis.

Importantly, this behavior is evidence of a continuous migration (diffusion) of triclosan from inner to the upper (surface) part of the agglomerate in order to compensate the concentration gradient generated by removing of such molecules from the surface to the nutrient agar.



In this respect, the total migration after 15 analysis cycles from quartz agglomerate substrates containing 500 ppm (302.75 µg) and 850 ppm (543.38 µg) of triclosan was determined by measuring the amount of triclosan molecules located at a sample depth of 1200 µm and 1250 µm, respectively.

This data fully supports the notion that triclosan migration takes place from inner to the upper part of the material and is in agreement with the results obtained in Sections 3.1 and 3.2.

The data was quantified in the below table:

TCS concentration (ppm)	Sample	TCS lost after 15 analysis (µg)	TCS total quantity in 3.56 g of quartz agglomerate (µg)	Difference (µg)	TCS lost after 15 analysis (%)	Released TCS in thickness (µm)
0	A	0.00	0.00	0.00	0.00	0
400	B	164.88	1,424.00	1.259.12	11.58	811
450	C	230.14	1,602.00	1.371.86	14.37	1.023
500	D	302.75	1,780.00	1.477.25	17.01	1.191
550	E	332.66	1,958.00	1.625.34	16.99	1.189
600	F	337.33	2,136.00	1.798.67	15.79	1.106
650	G	458.96	2,314.00	1.855.04	19.83	1.389
850	H	543.38	3,026.00	2.482.62	17.96	1.257

These results show the migration behavior of triclosan in the present quartz agglomerates.

Unfortunately, this test could not be replicated with an equivalent Appleton composite since the Appleton product is not available commercially and because Applicants do not have a license to reproduce the Appleton product in the laboratory.

In any case, in view of the comparatively superior performance (Sections 3.1, 3.2 and 3.3) for the lifespan of the antibacterial effectiveness in the present quartz agglomerates in comparison to the agglomerates from Appleton, it is clear that the present agglomerates of the above-noted application demonstrate unexpectedly superior properties over the agglomerates disclosed in Appleton, independent of the nature of the antimicrobial biocide incorporated (organic or inorganic).

As has been discussed and in my opinion, these differences can be explained in terms of the better dispersion of the biocidal additive in the present quartz agglomerate by a pre-dispersion in the resin binder prior to the conventional process.

I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these

statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 17th January 2011

Dr. Raúl Pozas Bravo